REMARKS

Status of the Claims

Claims 1 and 3-16 are currently pending in the present application. Claims 1, 4, 13, and 15 are amended. The claims are amended without prejudice or disclaimer. Claim 1 is amended to incorporate the elements of claim 2, now canceled. Claim 4 is amended to delete the dependency to canceled claim 2. Claim 13 is amended for clarity. Claim 15 is amended to cancel the term "optionally."

Claim 16 is new. Support for new claim 16 is found throughout the application as originally filed including, *e.g.*, in original claims 2 and 15. No new matter is entered by way of this amendment. Reconsideration is respectfully requested.

Effective Filing Date

The Examiner states that the effective priority date of the instant application is August 13, 2003, which is the filing date of the Japanese priority document.

Applicants note that the instant application is the national stage application of PCT Application No. PCT/JP2004/011237, which has an international filing date of August 5, 2004. The PCT application claims the benefit of priority of Japanese application No. 2003-293125, which was filed on August 13, 2003. Accordingly, the U.S. filing date of the instant application is August 5, 2004. However, Applicants may file an English translation of the priority document, *i.e.*, Japanese application No. 2003-293125, *e.g.* to antedate a reference or if requested by the Examiner, *see* 37.C.F.R. § 1.55

Objections to the Specification

The Examiner objects to the specification for omitting a reference to the PCT application. The specification is amended to reference the PCT application. Accordingly, withdrawal of the objection is requested.

The Examiner further objects to page 23 of the present application. The Examiner states that page 23 describes a drawing that does not correspond with the Figure numbering. The

specification is amended in accordance with the Examiner's requirements. Withdrawal of the objections is respectfully requested.

Abstract

The Examiner requests a clean copy of the abstract. Applicants submit herewith a clean copy of the Abstract on a separate page. Applicants have further corrected a grammatical error in the abstract. Withdrawal of the objection is respectfully requested.

Claim Objection

Claim 13 is objected to for an allegedly awkward recitation of "method of claim 1 subsequently to the step 2)...".

Claim 13 is amended according to the Examiner's suggestion. Withdrawal of the objection is respectfully requested.

Issue Under 35 U.S.C. § 112, Second Paragraph

Claim 15 is rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. Specifically, the Examiner states that the preamble of claim 15 specifies a method for obtaining a plant, while step 4 indicates that obtaining a plant from a transformed cell is optional.

Claim 15 is amended to cancel the term "optionally." Accordingly, Applicants believe the rejection is overcome and respectfully request withdrawal.

Provisional Non-Statutory Double Patenting Rejections

Claims 1, 4-9, and 12-15 are provisionally rejected on the ground of non-statutory obviousness-type double patenting as allegedly unpatentable over claims 1, 12, 14-15, 17-18, 20-21 and 23-24 of co-pending Application No. 10/089,695.

Further, claims 1, 4-9, and 12-15 are provisionally rejected on the ground of non-statutory obviousness-type double patenting as allegedly unpatentable over claims 22-29 of co-pending Application No. 10/089,696.

Claims 1 and 15 were amended to incorporate the subject matter of claim 2, which was not rejected. Applicants respectfully submit that the claims, as amended, are therefore not obvious in view of the above-cited applications. Accordingly, withdrawal of the rejections is respectfully requested.

ANTICIPATION

Rejections Under 35 U.S.C. § 102(b)

Hiei I

Claims 1, 4-6, 8-9, and 12-15 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by European Patent Application No. 1,306,440 to Hiei *et al.* The Examiner states that Hiei I describes all of the elements of the instant claims. The Examiner further alleges that the centrifugation step, as described in Hiei I, encompasses the pressurization step in the instant claims. According to the Examiner, an ordinary artisan would have recognized that centrifugal force inherently exerts pressure on the material to which it is applied.

As amended, claim 1 is directed to a method of introducing a gene into a plant material via Agrobacterium, comprising: 1) pressurizing the plant material, and then 2) infecting the plant material with an Agrobacterium, wherein pressurization is performed in the range of 1.7 atmospheres to 10 atmospheres. As noted above, support for "wherein pressurization is performed in the range of 1.7 atmospheres to 10 atmospheres" is found in claim 2, now canceled. Claim 2 was not rejected as being anticipated by Hiei I. Accordingly, independent claims 1 and 15 are novel over this reference. Dependent claims 4-6, 8-9, and 12-14, which incorporate the elements of independent claim 1 are also not anticipated by Hiei I. Further, new independent claim 16 also specifies that the pressurization is performed in the range of 1.7 atmospheres to 10 atmospheres. Accordingly, the instant claims are not anticipated by Hiei I. Withdrawal of the rejection is respectfully requested.

Hiei II

Claims 1, 4-9, and 12-15 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by EP 1,306,441 to Hiei *et al.*, ("Hiei II"). The Examiner states that Hiei II describes all of the elements of the instant claims. The Examiner further alleges that the centrifugation step described in Hiei II encompasses the pressurization step in the instant claims. According to the Examiner, an ordinary artisan would have recognized that centrifugal force inherently exerts pressure on the material to which it is applied.

As noted above, the instant claims are amended to specify "wherein pressurization is performed in the range of 1.7 atmospheres to 10 atmospheres." Also as noted above, support for this element is found, *e.g.*, in claim 2, now canceled, which was not rejected as allegedly anticipated by Hiei II. Accordingly, independent claims 1 and 15 are novel over this reference. Dependent claims 4-9, and 12-14, which incorporate the elements of independent claim 1 are also not anticipated by Hiei II. As noted above, new independent claim 16 also specifies that the pressurization is performed in the range of 1.7 atmospheres to 10 atmospheres. Accordingly, the instant claims are not anticipated by Hiei II. Withdrawal of the rejection is respectfully requested.

Cheng

Claims 1, 4-6, 10, and 13-15 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Cheng *et al.*, *Plant Cell Reports*, 1996, 16:127-132, ("Cheng"). According to the Examiner, Cheng teaches all of the elements of the instant claims.

As noted above, the claims are amended to specify "wherein pressurization is performed in the range of 1.7 atmospheres to 10 atmospheres." Also as noted above, support for this element is found, *e.g.*, in claim 2, now canceled. Claim 2 is not rejected as allegedly anticipated by Cheng. Accordingly, independent claims 1 and 15 are novel over this reference. Dependent claims 4-6, 10, and 13-14, which incorporate the elements of independent claim 1 are also not anticipated by Cheng. New independent claim 16 also specifies that the pressurization is performed in the range of 1.7 atmospheres to 10 atmospheres. Accordingly, the instant claims are not anticipated by Cheng. Withdrawal of the rejection is respectfully requested.

Knittel

Claims 1-3, 6, 10, and 13-15 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Knittel *et al.*, *Plant Cell Reports*, 1994, 14:81-86, ("Knittel"). Applicants respectfully traverse.

According to the Examiner, Knittel describe the transformation of sunflower tissue via the bombardment of the tissue with microparticles, propelled through gas at a pressure of ~8 bars (7.9 atmospheres).

In this context, Applicants submit that the relevant portion of Knittel is as follows:

> After 4 rinses with sterile water, they [tungsten microparticles] were suspended in TE buffer (Tris-HCl 10 mM, pH 8, EDTA 1mM) at 0.17 mg/ml, and 2 µl of this suspension were loaded onto the grid of a helium particle inflow gun **(Finer *et al.* 1992)**. Half apices, placed upright, were bombarded twice at a distance of 16 cm, **a vacuum of 28 mm Hg (0.95 bar), and a helium pressure of 8 bar.** *See* page 83 of Knittel, left column, lines 28-33, *emphasis added.*

Based upon the foregoing, the 8 bar pressure disclosed in Knittel applies to the helium gas pressure, which is used for bombardment of a helium particle. The plant tissue *per se* is under decompressed conditions, *i.e.*, a vacuum of 28 mm Hg (0.95 bar).

Applicants also submit herewith a copy of Finer *et al*, "Development of the particle inflow gun for DNA delivery to plant cells", *Plant Cell Reports*, 1992, vol. 11, pp. 323-328, ("Finer"), *i.e.* Exhibit A, cited in Knittel to support the above-described interpretation of this reference. Figure 1 of Finer depicts a graphic illustration of a particle inflow gun. The Figure shows that particles are bombarded by the pressurized helium gas from a syringe filter. The plant tissue is placed in a vacuum chamber, and, accordingly, is not pressurized.

In further support of the lack of pressurization, Finer states at page 325, left column, lines 9-41, "[a] vacuum of 28-30 in Hg was applied and the particles were discharged when the helium (at 40-80 PSI)...." "The particles were directly accelerated in the helium stream...", *see* page 325, under the heading Results and Discussion, Particle Inflow Gun Development:, lines 35-36. In addition, Finer further states that:

> The vacuum reduced the drag on the particles and lessoned tissue damage by dispersion of the helium gas prior to impact. The vacuum also contributed to the pressure differential, which may have been responsible for the efficient particle acceleration. *See* page 325, left column under the heading "Results and Discussion" and "Particle Inflow Gun Development", lines 42-44.

Accordingly, Knittel does not describe pressurization of plant tissue, as specified in the instant claims.

Further, Knittel describes using a helium inflow particle gun to bombard plant tissue. Accordingly, Knittel's method requires wounding of plant tissue.

In contrast, the claimed method does not generally cause or require injury of a plant tissue. The present invention is based on the finding that efficiency of gene transfer can be increased by applying appropriate pressure to a plant tissue before infection with *Agrobacterium*, which is independent of wounding (injury). As described in the present application, gene transfer efficiency is increased in comparison to a control (1.0 atmosphere), even when a low pressure of 1.4 is applied (2.4 atmosphere). Accordingly, the low pressure used in the claimed methods does not injure plant tissue.

In addition, "pressurizing the plant material", as described in the instant claims, is completely different from bombarding a plant using a helium inflow particle gun, which physically injures plant tissue, as described in Knittel. *See, e.g.*, paragraph [0017] of the instant application, which describes specific examples for performing the step of pressurizing plant material. Paragraph [0017] is reproduced below for the Examiner's convenience.

> [0017] Pressurization procedures can be performed by e.g., combining syringes, holding the syringes by a clamp and tightening the clamp to increase the pressure in the syringes. The pressurization force can be calculated from e.g., the loss of the volume of air in the syringes. Alternatively, pressurization may also be performed by 1) supplying a gas into a vessel containing a plant tissue from a compressor or the like to increase the inner pressure of the vessel, or 2) submerging a plant tissue contained in a bag or the like sealed against the outside air in a liquid to hydraulically pressurize it.

None of the above-described exemplary pressurizing steps would cause injury to a plant tissue. Accordingly, as noted above, the claimed method allows for increasing the efficiency of gene transfer by applying appropriate pressure to a plant tissue, independent of wounding (injury) the plant tissue.

Based upon the foregoing, the pressurizing step, as described in the instant claims, is not disclosed in Knittel. Further, the use of a helium inflow particle gun for bombardment of plant tissue is distinguishable from pressurization, which an ordinary artisan would have recognized from the present application does not encompass procedures that require plant wounding. The instant claims, which describe pressurization in the range of 1.7 atmospheres to 10 atmospheres, further distinguishes the claimed method from Knittel.

Accordingly, Applicants submit that the instant claims are not anticipated by Knittel. Withdrawal of the rejection is respectfully requested.

Pioneer Hi-Bred

Claims 1, 6, 10-11, and 13-15 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by European Patent Application No. 486,233 to Pioneer Hi-Bred ("Pioneer Hi-Bred").

Applicants note that Pioneer hybrid was not cited against claim 2, now canceled. As amended, claims 1 and 15 incorporate the elements of claim 2. Dependent claims 6, 10-11, and 13-14 and new independent claim 16 also specify that the pressurization step is performed in the range of 1.7 atmospheres to 10 atmospheres. Accordingly, the instant claims are not anticipated by Pioneer Hi-Bred. Withdrawal of the rejection is respectfully requested.

OBVIOUSNESS

Although the instant claims have not been rejected under 35 U.S.C. § 103(a) as allegedly obvious, Applicants would like to note that the instant claims are not obvious over any of the cited references, either alone or in combination. None of the cited references describe the pressurizing step of the instant claims, as would have been understood from the

present application by an ordinary artisan.

For example, Hiei I is the European national stage application of PCT Publication No. WO 02/012520, *i.e.* PCT Patent Application No. PCT/JP00/05213. PCT/JP00/05213 corresponds to Japanese Patent Laid Open Publication No. JP 2000-342256. Hiei II is the European national stage application of PCT Publication No. WO 02/012520, *i.e.*, PCT Patent Application No. PCT/JP00/05214, which thus corresponds to JP 2000-342253. JP 2000-342256 and JP 2000-342253, which thus correspond to Hiei I and Hiei II, respectively, are described in paragraphs [0040] and [0041] of the instant application. As noted in the present application, Hiei I discloses centrifugation of plant material and Hiei II discloses the combination of heat treatment and centrifugation treatment. Hiei I describes that centrifugation may be performed at accelerations of 100G to 250,000G, preferably 500G to 200,000G, and more preferably 1000G and 150,000G, *see* paragraph [0012] of Hiei I.

In contrast to Hiei I and Hiei II, the instantly claim method comprises "pressurizing" the plant material. As noted above, paragraph [0017] of the present application describes that pressurization procedures can be performed by, *e.g.*, combining syringes, holding the syringes by a clamp and tightening the clamp to increase the pressure in the syringes. Pressurization may also be performed by 1) supplying a gas into a vessel containing a plant tissue from a compressor, or the like, to increase the inner pressures of the vessel, or 2) submerging a plant tissue contained in a bag, or the like, or hydraulically pressurizing the tissue by placing the tissue in a liquid, where it is not exposed to air.

Based upon the foregoing, an ordinary artisan would have recognized that the pressurizing step, as described in the instant claims, *i.e.*, wherein pressurization is performed in the range of 1.7 atmospheres to 10 atmospheres, is not taught or suggested in Hiei I or Hiei II. At the time of the invention, an ordinary artisan would have recognized that plant tissues are transformed with a higher efficiency using *Agrobacterium*-mediated gene transformation after centrifugation. However, Applicants submit that a "centrifugation" step and a "pressurizing" step are completely distinguishable. At the time of the invention, an ordinary artisan would not have recognized that pressurizing plant tissue could improve the efficiency of *Agrobacterium*-mediated gene transformation from the disclosure in Hiei I or Hiei II regarding centrifugation.

Further, neither Cheng, Pioneer Hi-Bred, nor Knittel teach or suggest pressurization. Cheng teaches an *Agrobacterium*-mediated transformation based upon *wounding* of cultured embryogenic tissues with caborundum in a liquid phase, *see* page 127, left column, lines 4-7 of Cheng. On page 128 under the section entitled "Materials and Methods" under the subheading "Plant transformation and regeneration", the Cheng reference states that carborundum-wounded calli, were submerged in the culture of *A. tumefaciens* for 5 minutes.

Pioneer Hi-Bred describes the transformation of tobacco tissue via bombardment of the tissue with micro-particles propelled through gas. In particular, Pioneer Hi-Bred provides a transformation method in which plant tissues are first perforated with microprojectiles that do not carry genetic materials. The high velocity impact of dense particles on plant tissues would generate a wide array of microwounds, *see* page 2, lines 54-57 of Pioneer Hi-Bred.

As noted above, Knittel also describe transformation methods requiring wounding of the plant material.

In contrast, the instant claims describe a pressurization step, wherein pressurization is performed in the range of 1.7 atmospheres to 10 atmospheres. This step is not taught or suggested in the cited references. Unlike Cheng, Knittel and Pioneer Hi-Bred, the method for increasing gene transfer efficiency does not require wounding of the plant material.

Experimental Evidence

To further support Applicants' statements that the presently claimed invention does not require wounded plant material, Applicants submit herewith Exhibit B, *i.e.*, EXPERIMENTAL DATA (A).

Exhibit B demonstrates the results of a study to determine the extent of pressure-induced damage of plant materials using the claimed methods. Specifically, plant cells were treated with 10 atmosphere pressure or sonication. Cell death and cell survival were compared in the two populations. Cell survival and cell death was determined by phenosafranin staining, which is a method generally well-known in the art. As described in Exhibit A, more than 70% of cells treated with sonication for 2 seconds died. In contrast, cells treated with 10 atmosphere pressure for 15 minutes demonstrated a survival rate comparable to that of cells without any treatment. Accordingly, cell death due to pressure treatment was not observed.

Based upon the foregoing, injury (damage) to plant cells resulting from pressure treatment using the claimed methods is not significant in comparison to cells, which were wounded by sonication. The evidence shows that using a pressurization step encompassed by the instant claims, *e.g.*, under 10 atmospheres, does not result in wounding of the plant material.

As noted above, paragraph [0017] of the present specification, provides examples of how plant material may be pressurized according to the claimed methods, *e.g.*, combining syringes, holding the syringes by a clamp and tightening the clamp to increase the pressure in the syringes. Alternatively, the present application teaches that pressurization may also be performed by 1) supplying a gas into a vessel containing a plant tissue from a compressor or the like to increase the inner pressure of the vessel, or 2) submerging a plant tissue contained in a bag or the like sealed against the outside air in a liquid to hydraulically pressurize it.

Applicants submit that none of the above exemplary pressurization methods would cause injury to plant tissue. As noted above, the claimed methods allow for an increase in gene transfer efficiency via application of appropriate pressure to a plant tissue. However, injuring the plant tissue is not necessary.

Based upon the foregoing, Applicants submit that none of the cited references, either alone or combination, could have suggested the unexpected benefits of the instantly claimed invention. That is, a method for increasing gene transfer efficiency using pressurization at the described atmospheres, without causing injury to the plant material.

The unobviousness of the invention is further supported by the working examples, which provide experimental results of pressurization under the following conditions:

Example 1 (6)

2.4 atmospheres (+ 1.4 atmospheres), 15minutes
4.2 atmospheres (+ 3.2 atmospheres), 15 minutes
7.6 atmospheres (+ 6.6 atmospheres), 15 minutes

Example 1 (7)

7.6 atmospheres (+ 6.6 atmospheres), 1 seconds
7.6 atmospheres (+ 6.6 atmospheres), 3 seconds

7.6 atmospheres (+ 6.6 atmospheres), 5 seconds
7.6 atmospheres (+ 6.6 atmospheres), 60 seconds

Applicants submit herewith Exhibit C, *i.e.*, "EXPERIMENTAL DATA (B)." Exhibit C provides supplemental experimental data, which further demonstrates support for the pressure ranges and time periods of pressurization specified in the instant claims.

Time

Shorter times-6 atmospheres, 1 seconds
Longer times-8 atmospheres, 30 minutes

Pressure

Lower pressure-2 atmospheres, 15 minutes.
Higher pressure-10 atmospheres, 15 minutes

Applicants further direct the Examiner's attention to Figure 1 of Exhibit C, which depicts treatment of plants at 6 atmospheres for **1 second.** This treatment resulted in GUS expression which was more than three times that of the control, which did not receive the pressurization treatment. That is, a pressurization treatment of only 1 second was sufficient to promote the introduction of a gene into a plant material. If required by the Examiner, Applicants are further able to provide experimental data, which demonstrates that even shorter time periods are effective, *i.e.*, less than 1 second.

Applicants thus submit that the claims are novel and non-obvious over the cited references. Accordingly, a notice of allowance is respectfully requested.

CONCLUSION

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Linda T. Parker, Reg. No. 46,046, at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Dated: SEP 02 2009

Respectfully submitted,

By _____
Gerald M. Murphy, Jr.
Registration No.: 28,977
BIRCH, STEWART, KOLASCH & BIRCH, LLP
8110 Gatehouse Road
Suite 100 East
P.O. Box 747
Falls Church, Virginia 22040-0747
(703) 205-8000
Attorney for Applicant

Attachments: Exhibits A-C
Appendix with clean copy of Abstract